Ex A

Attorney's Docket No.: 10274-107001 / A003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Chicheportiche, et al. Art Unit : 1635
Serial No. : 09/245,198 Examiner : R. A. Schnizer
Filed : February 5, 1999
Title : A TUMOR NECROSIS FACTOR RELATED LIGAND

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SECOND DECLARATION OF JEFFREY BROWNING, PH.D.

UNDER 37 C.F.R. § 1.132

I, Jeffrey Browning, Ph.D., hereby declare and state as follows:

1. I am one of the named co-inventors of the above-identified patent application. I currently hold the position of Distinguished Investigator at Biogen Idec, one of the co-assignees of this application. My resume and qualifications are more fully described in ¶ 1 of the (first) Declaration of Jeffrey Browning, Ph.D., executed on February 6, 2004 and mailed to the Office on February 25, 2004.

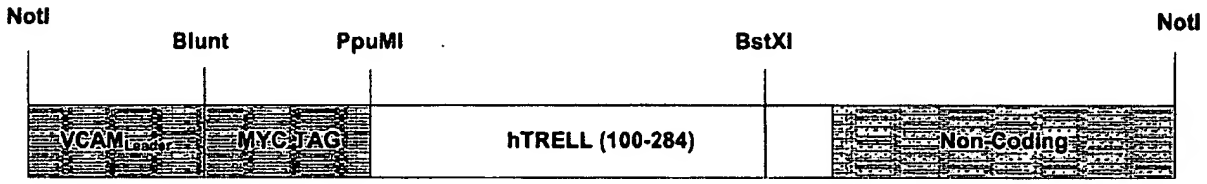
2. I understand that during the telephone conference dated May 25, 2005 the Examiner inquired about the protein encoded by the construct described at page 34 of the specification. In the Interview Summary dated May 27, 2005, the Examiner requested that I clarify "what fragment of the human protein had actually been expressed, (i.e., what fragment is encoded by the PpuMI-NotI construct discussed at page 34 of the specification)."

3. The specification states, within the paragraph bridging pages 34-35, that:

> The following DNA fragments were isolated, a Not1/blunt fragment encoding the VCAM leader and a pair of oligonucleotides encoding the myc tag (5' blunt, 3' PpuM1 site) which have been described, a 0.45 kb PpuM1/BstX1 fragment of TRELL and a 0.65 BstX1/Not1 fragment of TRELL. The four fragments were ligated into a Not1/phosphatased pBluescript vector. The Not1 insert from this vector was transferred into the pFastBac1 vector (GibcoBRL) and used to generate recombinant baculovirus. Soluble TRELL was prepared by infecting HiFive™ insect cells at a MOI of 10 and the medium was harvested after 2 days.

This construct is also described in Chicheportiche et al., (1997) *J. Biol. Chem.* 272:32401-32410 (Exhibit A), particularly at page 32402, left column bottom at the paragraph titled, "Expression of Recombinant hTWEAK protein." TWEAK is the current name for the protein described as TRELL in the application.

4. A general schematic of the NotI insert in the construct is indicated below:



A detailed view of the sequence at the PpuMI site (an enzyme which cleaves leaving a 5' overhang by cleaving RG^GWCCY) is shown below.

	R	D_{100}	Q_{101}		Position in SEQ ID NO:4	
Oligos encoding Myc Tag	5' CTG	GAC	CAG	. . .	3'	PpuMI – BstXI fragment of TRELL
	3' GAC	CTG	GTC	. . .	5'	

As seen above, the human TRELL coding sequence (right side) was joined to annealed oligonucleotides encoding the Myc Tag (left side) using the overhangs from a PpuMI restriction site (nucleotides 295-301 of SEQ ID NO:3) in the TRELL coding sequence. The codon within the overhang encodes amino acid 100 of SEQ ID NO:4. This amino acid is aspartic acid (D). Ligation of the PpuMI-BstXI fragment to the BstXI-NotI fragment produced a sequence that encodes amino acids 100 to 284, the C-terminus of human TRELL. Accordingly, the protein encoded by the construct described on page 34 of the specification is a fusion of the VCAM leader, the myc epitope tag and a fragment of human TRELL that corresponds to amino acids 100 to 284 of SEQ ID NO:4.

5. The application also discloses at least a functional part of murine TRELL. An alignment of a murine TRELL amino acid sequence to the human TRELL amino acid sequence is shown in FIG. 1 of the application. The alignment is complete within the region defined by the fragment of human TRELL that was expressed by the constructed described on page 34 of the specification. The corresponding fragment of murine TRELL is easily identifiable from the alignment.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Jeffrey Browning, Ph.D.

Cambridge, MA

Date: 6-23-05